

# Clinical Features, Pathogenesis, and Treatment of Large Granular Lymphocyte Leukemias

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## Abstract

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Large granular lymphocytes (LGLs) are large lymphocytes with azurophilic granules in their cytoplasm. LGLs are either natural killer (NK) cells or T lymphocytes. Expansions of the LGLs in the peripheral blood are seen in various conditions, including three clonal disorders: T-cell LGL (T-LGL) leukemia, chronic lymphoproliferative disorders of NK cells (CLPD-NK), and aggressive NK-cell leukemia (ANCL). However, the monoclonal and polyclonal expansion of LGLs has been associated with many other conditions. The present article describes these LGL disorders, with special emphasis on the clinical features, pathogenesis, and treatments of the three above-mentioned clonal disorders.

**Key words:** LGL, T-LGL leukemia, chronic lymphoproliferative disorders of NK cells, aggressive NK-cell leukemia

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## Introduction

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Large granular lymphocytes (LGLs) are large lymphocytes with round or reniform nuclei, a broad cytoplasm, and azurophilic granules in their cytoplasm (Fig. 1) (1). They account for 10-15% of the peripheral blood mononuclear cells. Most of the normal LGLs in the peripheral blood are natural killer (NK) cells, while some are T lymphocytes (2). Monoclonal or polyclonal LGL expansions are found in patients with various conditions. According to the current World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues, clonal LGL expansions are divided into three disorders (3): T-cell LGL (T-LGL) leukemia, chronic lymphoproliferative disorders of NK cells (CLPD-NK), and aggressive NK-cell leukemia (ANCL) (Fig. 2-1 to Fig. 2-4). T-LGL leukemia is the most common disorder, followed by CLPD-NK; ANCL is rare.

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## Case Description

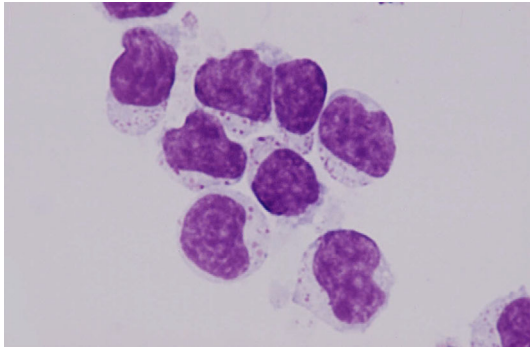
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A 40-year-old single physical education teacher presented to the author's clinic with T-LGL leukemia and pure red cell aplasia (PRCA). He had been diagnosed with CD3+CD4-

CD8- T-LGL leukemia at another hospital 7 years previously. His hemoglobin level was 8 g/dL, and a bone marrow examination revealed 7% erythroblasts. Cyclophosphamide was recommended, but he refused it due to the possibility of infertility (4). Three years later, cyclosporine was administered, but was subsequently discontinued due to renal damage. One year later, the patient's hemoglobin level dropped to 2 g/dL, a bone marrow examination revealed 3% erythroblasts, and he became blood transfusion-dependent. The patient was diagnosed with PRCA and was referred to the author's clinic.

On a physical examination, the patient was pale, without apparent lymphadenopathy or hepatosplenomegaly. A laboratory analysis revealed the following: hemoglobin, 3 g/dL; reticulocytes, 1.3%; WBC count,  $3.4 \times 10^9/L$  with 68.2% lymphocytes. Most of the lymphocytes were "small" lymphocytes (approximate diameter: 12.2  $\mu m$ ) with numerous azurophilic granules in their narrow cytoplasm. The lymphocytes were CD3+CD4-CD8-CD16-CD56-CD57-, T-cell receptor (TCR) $\alpha\beta$ - and TCR $\gamma\delta$ +. The TCR  $J\gamma$  and TCR  $C\beta 1$  genes displayed monoclonal rearrangement. *STAT3* Y640F, D661Y or *STAT5b exon16* mutations were not found.

Cyclosporine was restarted, but was discontinued again due to renal damage. Methotrexate was administered and



**Figure 1.** LGLs isolated from a normal individual.

then discontinued due to hepatotoxicity. The patient was then treated with azathioprine, which was also discontinued due to hepatotoxicity. After storing the patient's sperm, oral cyclophosphamide (100 mg, daily) was administered for two weeks; the dose was then reduced to 50 mg daily. The patient's reticulocyte count began to increase one month later, and his anemia disappeared eight months later. Cyclophosphamide was discontinued after the TCR genes were confirmed to show germ-line configurations by Southern blotting. In total, it was administered for 10 months. One year has passed since treatment was discontinued, and no relapse has been detected. As a physical education teacher, he is now able to run with his students.

### The Clinical Features of T-LGL Leukemia

T-LGL leukemia is a rare lymphoproliferative disorder that usually follows an indolent clinical course. As shown in Table, it typically develops around 60 years of age (median), with males and females equally affected (females may be slightly more affected). T-LGL leukemia is associated with various types of cytopenia. In Western countries, severe neutropenia with infection is often seen (5-13). In contrast, neutropenia is not as common in Japan and China, while severe anemia (including PRCA) is more frequently found (14-18). In Western countries, T-LGL leukemia is commonly associated with rheumatoid arthritis and Felty's syndrome (5-13); in Asia, association with these diseases is not common (14-18).

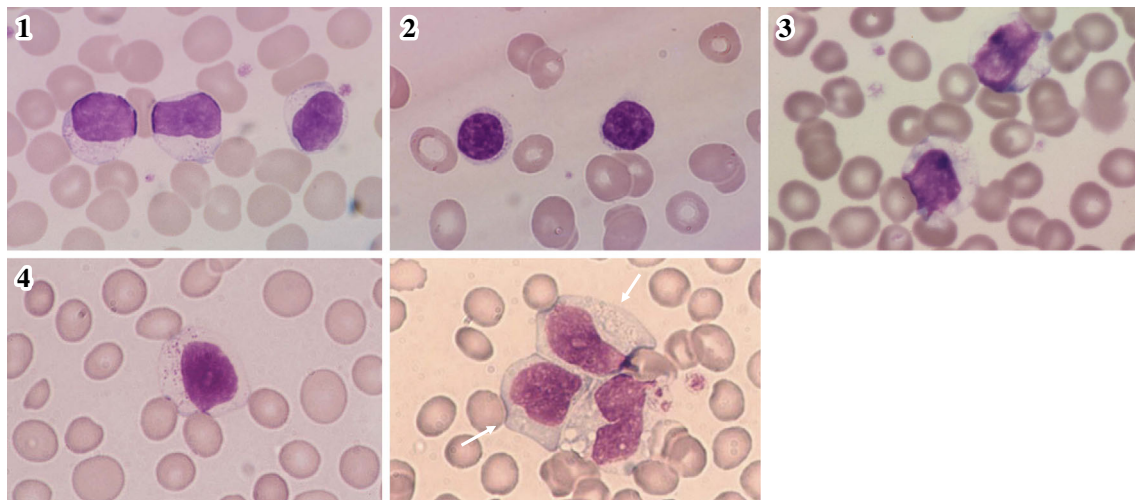
The surface phenotype of T-LGL leukemia is mostly CD3+CD4-CD8+, and less often CD3+CD4-CD8-, CD3+CD4+CD8-, or CD3+CD4+CD8+. The normal counterpart of CD3+CD4-CD8+ leukemic cells is thought to be *in vivo*-primed effector-type cytotoxic T lymphocytes (CTLs) of unknown antigen specificity, because T-LGLs are mostly CD45RA+CD27-CD28-CD57+/-CD94+NKG2+ (19, 20), contain perforin and other cytotoxic molecules (21, 22), and exhibit strong cytotoxicity *in vitro* when anti-CD3 antibodies are added to cultures of CD3+ LGLs and Fcγ receptor-bearing target cells (23), and when anti-CD3 × anti-CD10 bispecific antibodies are added to the cultures of CD3+ LGLs and CD10+ target cells (24). CD3+CD4+CD8-/+ leukemic cells

are considered to be derived from T-LGLs with the CD4+CD56+TCRαβ+ phenotype found in small quantities in normal individuals (25). CD3+CD4-CD8- T-LGL leukemia is almost always of the TCRγδ+ phenotype, which is present in small amounts in the normal peripheral blood. Its clinical features are not very different from those of the TCRαβ+ phenotype (26-29). CD56+ T-LGL leukemia has been reported to follow an aggressive clinical course (30, 31); however, the presence of the CD56 antigen does not always indicate an aggressive clinical course. In our series, the CD56 antigen was detected in 4 of 21 cases of indolent T-LGL leukemia. In contrast, the one patient with aggressive T-LGL leukemia was negative for the CD56 antigen (14-16).

### The Diagnostic Criteria and Terminology of T-LGL Leukemia

The diagnostic criteria of T-LGL leukemia are somewhat confusing. In the 2008 WHO Classification (3), T-LGL leukemia is defined by a persistent (>6 months) increase in the number of peripheral blood LGLs (usually  $2\text{-}20 \times 10^9$  LGLs/L), without a clear cause. However, patients with  $<2 \times 10^9$  LGLs/L of clonally expanded LGLs are often seen, and the clinical features of these patients have been reported to be similar to those in with  $>2 \times 10^9$  LGLs/L (32). The duration of LGL persistence is not necessarily required for the diagnosis, because patients with severe anemia or neutropenia must be treated immediately without observing them in a drug-free state for six months or more (15, 16). It is the authors opinion that whenever a patient presents cytopenia (either anemia or neutropenia) of an unexplained etiology, the demonstration of the clonal expansion of LGLs should be sufficient to make a diagnosis of T-LGL leukemia, irrespective of whether more than  $2 \times 10^9$ /L LGLs are present (15, 16). In the absence of cytopenia, we must carefully differentiate whether a reactive and transient process is being observed. The clonality of the LGLs is detected by the Southern blotting of the TCR genes, a PCR of the TCR genes, or by monoclonal antibodies against TCR Vβ gene repertoires. In daily clinical practice, Tanahashi et al. (33) recommended a simplified way of identifying the LGL leukemia of T- and NK-cell lineages when both of the following criteria are fulfilled: lymphocytes account for >52% of the WBC, and when >50% of the lymphocytes are LGLs.

In normal individuals, the absolute numbers of LGLs in the peripheral blood have been reported to be  $223 \pm 99/\mu\text{L}$  (mean±SD) (5),  $198 \pm 112/\mu\text{L}$  (34), and  $210 \pm 20/\mu\text{L}$  (35). The number in males is significantly higher than that in females (35). The author's examination revealed that the absolute numbers of peripheral blood LGLs in normal Japanese individuals were  $630 \pm 261/\mu\text{L}$  in males (n=20), and  $350 \pm 176/\mu\text{L}$  in females (n=20), with the number in males being significantly higher (14). In an examination of 35 normal Japanese individuals, Ishida et al. (personal communication) found that the number was  $320 \pm 265/\mu\text{L}$ . It is not clear whether the difference in the absolute LGL counts between



**Figure 2.** Peripheral blood smears of T-LGL leukemia, CLPD-NK, and ANKL patients. 1: T-LGL leukemia. 2: T-LGL leukemia, small-cell type. 3: CLPD-NK. 4: ANKL (the arrows indicate ANKL cells). T-LGL: T-cell large granular lymphocyte, CLPD-NK: chronic lymphoproliferative disorders of natural killer cells, ANKL: aggressive NK-cell leukemia

**Table.** The Clinical Features of T-LGL Leukemia and CLPD-NK Reported in Asian and Western Countries.

Reference	15, 16		18	8		10		11	12	13	
	T	NK	T	T	NK	T	NK	T	T	T	NK
T or NK											
Number of patients	35	10	22	129	11	44	14	29	26	201	28
Median age	60	58	47	7	39	63	67	57	58	59	58
Male/female	16/19	4/6	14/8	57/71	6/5	22/22	7/7	12/17	13/13	90/111	14/14
Splenomegaly (%)	11	10		50	91	35	0	66	23	24	25
Hepatomegaly (%)	3	0		23	64			10	12	10	14
Adenopathy (%)	3	0		1	27	5	0	10	4	6	7
Rheumatoid Arthritis (%)	6	0		28	0	20	14	38	15	17	11
Neutropenia (%)	54	20	36	84	64	52	69	77		61	48
<math>0.5 \times 10^9/L</math> (%)	6	10	0	48	18	41	46	69	23	26	16
Anemia (%)	80	30	86	49	1	89	77	77		24	25
Hb<math><8.0</math> g/dL (%)	46	0	50			36	31		15	7	4
Thrombocytopenia (%)	6	10	23	19	75	36	15	45	35	19	8

Our studies (15, 16) and Kwong's study (18) are from Asia. The others (8, 10-13) are from Western countries. T-LGL: T-cell large granular lymphocyte, CLPD-NK: chronic lymphoproliferative disorders of natural killer cells

Japanese individuals and individuals from other backgrounds stems from differences between races and/or environmental factors. The French study group accepted, with some exceptions, the diagnostic criteria of the peripheral expansion of LGLs ( $>0.5 \times 10^9/L$ ) for a period of  $>6$  months, excluding transient or reactive LGL proliferation (13).

Historically, Brouet et al. (36) first described the expansion of LGLs in some patients with T-cell chronic lymphocytic leukemia in 1975. McKenna et al. (37) analyzed four such cases, and considered the process to be a distinct clinicopathological entity in 1977. Since then, similar case descriptions have been accumulated. LGL leukemia has had various names. Leukemia of LGL was first named by Loughran et al. (5, 6), while the name T-LGL leukemia has been accepted worldwide, particularly since it was proposed

in the 2001 and 2008 WHO classifications. Other names have included T $\gamma$ -lymphoproliferative disease (38), lymphoproliferative disease of granular lymphocytes (7), and granular lymphocyte-proliferative disorders (14-16). Some of the names use the term leukemia, while others do not. The reason why the term leukemia is not used is because it is unclear whether T-LGL leukemia represents a true lymphoid malignancy or a self-limited clonal proliferation. Because the number of LGLs stays stable over a period of time, and because spontaneous regression can occur, most T-LGL leukemia patients do not seem to have leukemia. Similarly to the situation of monoclonal gammopathy of undetermined significance (MGUS), the alternative name of "T-cell clonopathy of undetermined significance" has been proposed (9). The reason why the term granular lymphocytes (GLs), but

not LGLs, is sometimes used because some patients have small GLs (rather than large GLs) as described above in the Case Description. Tanahashi et al. (39) compared the diameter of normal GLs and found that the GLs from most patients with LGL leukemia were  $\geq 14 \mu\text{m}$ , while 6 out of the 26 patients with LGL leukemia showed GLs of  $\leq 12.5 \mu\text{m}$ . This small subtype was recognized among younger patients and was associated with *STAT3* mutations (39). As shown in Fig. 2, most of our cases exhibited typical LGLs, while some exhibited small GLs.

Lymphocytes are described as “large” when they are more than twice the size of erythrocytes in diameter. Lymphocytes that are less than twice the size of erythrocytes are considered to be “small” (40). Then what is the diameter of T-LGL leukemia cells? Some are more than twice the size of erythrocytes, but many are less twice the size. Strictly speaking, the term LGL cannot be uniformly applied. However, it is not known whether the size of LGLs changes after forced drying when peripheral blood films are made. In Japan blood films are dried with cold air using a dryer. In Western countries, to the best of the author’s knowledge, films are dried naturally without using a dryer. We have to compare the size differences between these two methods.

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### LGL Expansions in Various Conditions

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The monoclonal expansion of nonmalignant B cells occurs frequently in elderly individuals. This is thought to be a consequence of the chronic stimulation of B cells by self-antigens or microbial antigens over the patient’s lifetime. The similar clonal expansions of T cells occur in elderly individuals. The clonal CD3+CD8+CD28-TCR $\alpha\beta$ + cell populations are frequently found in normal subjects of  $>65$  years of age; in such patients, they represent up to 37.5% of all CD8+ cells (41). CD3+CD8+CD28- cells have been shown to lack proliferative responses to CD3/TCR stimuli and to display high CTL activity. Thus, it is possible that repeated exposure to major histocompatibility complex (MHC) class I-restricted antigens results in the clonal accumulation of end-stage mature CTLs. However, it was not described whether the morphology of these CTLs was similar to that of LGLs. It is likely that these clonal CD8+ CTLs respond to the same antigenic stimuli, regain their proliferative capacity, and result in T-LGL leukemia.

LGL expansions are found in various conditions. Viral infections, including cytomegalovirus (CMV), have been reported to induce transient or persistent clonal CD3+CD4+ or CD3+CD8+ T-LGL expansions (42), and CMV-expanded T-LGLs have been proven to recognize CMV antigens (43). Serum reactivity to human T-cell leukemia virus (HTLV) type 1 proteins was described in LGL leukemia patients (44). Forty-four percent of 53 patients had anti-HTLV antibodies (45). However, HTLV-1 to -4 and their related viruses have not been proven to be causative agents of LGL leukemia (45).

The persistent elevation of monoclonal or oligoclonal

CD3+CD8+CD57+TCR $\alpha\beta$ + CTL or T-cell LGLs has been reported in a significant proportion of recipients following allogeneic hematopoietic stem cell transplantation (HSCT) (46-49). These patients tend to have an overall survival advantage with a lower incidence of relapse, and a higher incidence of graft-versus-host disease and CMV reactivation; this implies that LGLs of donor origin may have a role in targeting the recipient’s tumor cells and normal tissues as well as CMV. The absence of *STAT3* gene mutations, and the declining longitudinal kinetics of LGLs in the late period support their benign nature (49). The clonal expansion of CD3+CD8+CD57+ LGLs has been reported after autologous HSCT (50, 51). The number of infections or the clinical response to autologous HSCT did not appear to be correlated with the degree of the LGL expansions, and the expansions were indolent, were not associated with cytopenias, and did not require therapy. Studies are required to determine the functions of expanded T-LGLs. Interestingly, LGL expansions have also been reported in solid organ transplant recipients (52). A significant number of heart and renal transplant patients developed clonal CD3+CD57+ T-LGL expansions. None of the patients developed neutropenia or had active donor organ rejection, and most patients did not receive therapy for T-LGL expansion. Although the details were not shown, NK-cell expansions were also found in most of the patients (52).

Some patients with chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia who are treated with the tyrosine kinase inhibitor dasatinib, but not with other tyrosine kinase inhibitors, show significant LGL expansions [reviewed in (53)]. This phenomenon was first described by Mustjoki et al. (54) and Kim et al. (55), and many reports thereafter. LGL expansions begin as early as two hours from the start of therapy, peak at 3 months, and persist throughout the therapy. The cessation of dasatinib treatment induces rapid decline of the LGL counts, almost to the baseline levels. Expanded LGLs are reported to be CD3+CD8+ monoclonal or polyclonal T cells, or NK cells. LGL lymphocytosis is often accompanied by fevers, colitis, and pleural effusions, which suggests that these LGLs are involved in the aberrant immune response. LGLs may participate in the elimination of residual leukemic cells, and LGL expansions are associated with excellent, long-lasting therapeutic responses in dasatinib-treated patients. LGL expansions may also participate in CMV inactivation.

T-LGL expansions are found in association with various B-cell dyscrasias, including MGUS, chronic lymphocytic leukemia, follicular lymphoma, and polyclonal hyper- or hypo-gammaglobulinemia (56, 57). The co-association of the B-cell pathology with T-LGL suggests that either a common antigen drives the clonal B and T cells, or that a B-cell abnormality could serve as the stimulus for T-LGL expansion as a form of anti-tumor surveillance.

## The Molecular Pathogenesis of T-LGL Leukemia

Leukemic LGLs were found to display high levels of activated signal transducer and activator of transcription 3 (STAT3), and the treatment of leukemic LGLs with the JAK-selective tyrosine kinase inhibitor AG-490 induced apoptosis with a corresponding decrease in STAT-DNA binding activity (58). Recently, recurrent somatic mutations in the Src homology (SH) 2 domain of the *STAT3* gene have been found in 27-40% of patients with T-LGL leukemia (59, 60) and 30% of patients with CLPD-NK (60), leading to the constitutive activation of STAT3 and the dysregulation of genes downstream of STAT3. These gain-of-function mutations enhance the transcriptional activity of the mutated proteins. Patients with *STAT3* mutations presented with neutropenia and rheumatoid arthritis more frequently than patients without these mutations (59), and patients with symptomatic disease and a history of multiple treatments (60). The association between PRCA and neutropenia with *STAT3* gene mutations was also reported in Asia (61, 62). Activating mutations in the SH2 domain of the *STAT5B* gene were identified in 2% of LGL leukemia patients (63). The clinical course of the disease in patients with the *STAT5B* N642H mutation was aggressive and fatal, and was clearly different from typical LGL leukemia (63). Activating *STAT5B* mutations were also found in 6 of 11 cases (55%) of CD4+CD8-/+TCR $\alpha\beta$ + T-LGL leukemia, whereas *STAT5B* mutations were very rare among patients with CD4-CD8+ T-LGL leukemia or CLPD-NK (25). The disease course in CD4+ T-LGL leukemia cohort was indolent, and none of the patients with *STAT5B* mutations required therapy during the observation period (25). *STAT3* and *STAT5B* mutations can be used as molecular markers for the diagnosis of LGL leukemia, and present novel therapeutic targets for STAT3 and STAT5b inhibitors, which are currently in development.

Other than the JAK/STAT pathways, multiple cell survival pathways, including sphingolipid signaling, RAS/MEK/ERK, and SFK/PI3K/Akt, have been found to be constitutively activated in LGL patients [reviewed in (64)]. A biological approach identified IL-15 and platelet-derived growth factor as master survival signaling switches that may have a profound effect on all known deregulations in T-LGL leukemia. The Fas-mediated apoptotic pathway is impaired, and this may support the accumulation of LGLs (65).

Taken together, the above findings strongly support the role of autologous or foreign antigenic stimulation as an initial event. This would lead to the expansion of fully differentiated effector/cytotoxic LGLs, which are not eliminated due to the impairment of the apoptotic pathway. The acquisition of *STAT3/STAT5B* mutations might occur late in the natural history of the disease, certainly later than the establishment of clonality (66).

## The Pathogenesis of Associated Cytopenias

Anemia, neutropenia, and less often thrombocytopenia are associated alone or in combination with T-LGL leukemia (Table). Anemia develops from the T-LGL-mediated suppression of erythroid progenitor cells (26, 38). Whether T-LGLs directly kill these progenitor cells or suppress erythropoiesis by means of humoral mediators is an intriguing question. In a patient with CD3+CD4-CD8-TCR $\gamma\delta$ + T-LGL leukemia, it was shown that the patient's LGLs directly killed erythroid progenitor cells (67). These LGLs, which express killer-cell inhibitory receptors, were shown to be able to kill target cells in an MHC-unrestricted manner, but their cytolytic activity was inhibited by signaling through killer-cell inhibitory receptors when target cells expressed HLA class I antigens. However, because the expression of HLA class I antigens on erythroid progenitor cells is physiologically downregulated, the T-LGLs could lyse erythroid progenitors. Immature CD34+ progenitors and myeloid cells express HLA class I antigens and they were not lysed by the T-LGLs. Regarding the pathogenesis of neutropenia, T-LGLs express Fas ligand (FasL), and mature neutrophils express Fas. Fas-FasL system-mediated neutrophil apoptosis is highly likely to explain the cause of neutropenia (reviewed in 68). Several studies have indicated the presence of anti-neutrophil autoantibodies in the serum of some patients, but in most of these cases the detected antibodies were anti-HLA antibodies, rather than true anti-neutrophil autoantibodies, and it is unlikely that anti-neutrophil autoantibodies cause neutropenia (68).

CD3+CD8+ T-LGL expansions are found in bone marrow failure syndromes, such as aplastic anemia (AA) (69, 70), paroxysmal nocturnal hemoglobinuria (PNH) (70), and myelodysplastic syndromes (MDS) (71, 72). The frequency with which T-LGLs and bone marrow failure syndromes co-exists suggests an etiological relationship rather than simple coincidence. In a large cohort of 367 patients with MDS and 140 patients with AA (73), *STAT3*-mutated clones could be found, not only in the known LGL-concomitant cases, but also in a small proportion of unsuspected cases (AA, 7%; and MDS, 2.5%). AA patients with *STAT3* mutations showed an interesting trend toward a better response to immunosuppressive therapy; an association with the presence of human leukocyte antigen-DR15 was also found. MDS patients harboring *STAT3*-mutated clones showed a lower degree of bone marrow cellularity and a higher frequency of developing chromosome 7 abnormalities. *STAT3*-mutated LGL clones may facilitate the persistent dysregulation of autoimmune activation, which is responsible for the primary induction of bone marrow failure in a subset of AA and MDS patients. Bone marrow failure patients and LGL leukemia patients have similarly elevated levels of antibodies to BA21 protein, which are located in the transmembrane region of the HTLV-1 envelope (74). This suggests that LGL leukemia, AA, MDS and PNH might share a common

pathogenesis. LGL expansions are frequently found in patients with cytopenia of unknown etiology and should be thoroughly investigated in this patient group (75).

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### The Treatment of T-LGL Leukemia

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Because the clinical course of most patients with T-LGL leukemia is indolent, it is not always necessary to treat this disease. The patients who require treatment include those with symptomatic or transfusion-dependent anemia, and those with severe neutropenia (absolute neutrophil count  $<500/\mu\text{L}$ ) or moderate neutropenia (absolute neutrophil count  $>500/\mu\text{L}$ ) with recurrent infections. There is no standard treatment. Immunosuppressive therapy with agents including cyclophosphamide, methotrexate, cyclosporine and steroids have been used.

For patients with T-LGL leukemia who have severe anemia, the author prefers to use cyclophosphamide as a first-line therapy. This preference is based on a 1997 study in which the author was involved, in which hematological complete remission was obtained in all of the seven patients treated with cyclophosphamide (76). Five of the seven patients were in molecular complete remission, and did not experience a relapse for at least one year after the discontinuation of cyclophosphamide. Cyclophosphamide was initiated at a daily dose of 100 mg, orally. The dose was reduced to 50 mg per day after two weeks. For Japanese patients, a continued daily dose of 100 mg seems to be excessive. Cyclophosphamide was administered until the absolute lymphocyte count fell to  $<1,000/\mu\text{L}$  and the TCR genes showed germ-line configurations. Hematological responses were first observed after 8 weeks of treatment, with the patients obtaining hematological complete remission after six months. Similar results describing the effectiveness of cyclophosphamide have been reported from Western countries (77, 78) as well as from Japanese retrospective nationwide study (79). The treatment is continued until TCR genes are in germ-line configurations; however, because the complications of infertility and MDS/acute myeloid leukemia are dependent on the cumulative cyclophosphamide dose and the length of exposure, the duration of cyclophosphamide treatment should be as short as possible. It has not been shown, however, whether TCR Southern blotting is sufficient to confirm the absence of clonality, or whether the absence of minimal residual disease should be confirmed by a PCR. Considering the presence of small amounts of clonal T lymphocytes in old healthy individuals, it may not be necessary to obtain a PCR-based complete remission.

In patients with severe neutropenia, cyclophosphamide is also effective (78). Methotrexate has long been used as a first-line therapy for T-LGL leukemia with anemia or neutropenia (80). A prospective multicenter phase II study showed that the overall response rate to methotrexate was 38%, and the patients who failed methotrexate therapy and who were subsequently treated with cyclophosphamide showed an overall response rate of 64% (81). Prospective

studies to compare cyclophosphamide and methotrexate are required. Successful responses to purine analogs have been reported, but the numbers of patients treated with these agents are still very low.

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### Chronic Lymphoproliferative Disorders of NK Cells (CLPD-NK)

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CLPD-NK, which was proposed as a provisional entity in the 2008 WHO Classification (3), is characterized by the chronic expansion of mature-looking CD3-CD56+/-CD16+ NK cells in the peripheral blood (82-85). The condition develops at approximately 60 years of age (median), and males and females are equally affected (Table). In most cases, patients present with a chronic indolent course without cytopenias or symptoms, and treatment is not required. The disease is diagnosed when  $\geq 2 \times 10^9/\text{L}$  NK cells are present in the peripheral blood for at least 6 months, without a clearly identified cause (3). The number of NK cells may remain stable without treatment or show spontaneous regression.

Considering the role of NK cells in innate immunity, some etiological agents may stimulate and expand a subset of the NK-cell population (86). The possible association between chronic viral infections and skewed NK-cell proliferation, as evidenced by the skewed expression of killer immunoglobulin-like receptors (KIR), has been described (86, 87). Such common foreign antigens may also stimulate T-cell LGLs, thereby leading to both disorders (66), as shown in a recent report, which described the detection of monoclonal T populations in patients with KIR-restricted CLPD-NK (88). Thus T-LGL leukemia and CLPD-NK are likely to represent two sides of the same coin, although they appear to be distinct, separate disorders (66).

Rare cases transform to ANKL. Patients with Epstein-Barr virus (EBV)-positive NK cells tend to transform. These EBV-positive patients usually have chronic active EBV infection, hypersensitivity to mosquito bites or hydroa vacciniforme, and should be carefully followed for the emergence of clonally expanding NK cells (83, 89-91). The clonality of EBV-positive NK cells can be examined by Southern blotting using a probe recognizing the EBV terminal repeat. A clonal expansion of NK cells is noted in a substantial number of such patients, which indicates that patients with EBV-associated CLPD-NK may develop ANKL or extranodal NK-cell lymphoma (83, 89-91).

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### Aggressive NK-cell Leukemia (ANKL)

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ANKL was originally reported by Fernandez et al. (92) and Koizumi et al. (93), and was further described by Imamura et al. (94), Song et al. (95) and Suzuki et al. (96). Patients are affected at a relatively young age (median: 42 years). There is no sex predilection (or a slight male predominance). It seems to be highly prevalent in Asian people.

Fever, hepatosplenomegaly and lymphadenopathy are common. The clinical course is rapidly progressive, and the prognosis is poor with an overall survival of 2 months (96, 97). Liver dysfunction, disseminated intravascular coagulation, and hemophagocytic syndrome are often seen during the course of the disease, particularly at the terminal stage. This disease is characterized by the presence of NK cells of a slightly immature-looking morphology, mainly in the peripheral blood, bone marrow, liver and spleen. A diagnosis is made when the following findings are fulfilled (83, 85, 97, 98): (i) morphologically, slightly immature-looking large lymphocytes, with a broad, pale cytoplasm and azurophilic granules, and somewhat fine nuclear chromatin and occasional nucleoli, are present in the peripheral blood and/or bone marrow; (ii) immunophenotypically, these lymphocytes are CD3-CD56+CD16-/CD57-; and (iii) genetically, they have germ-line configurations of the TCR and IGH genes. The ANKL cells appear slightly immature in their morphology, but the presence of the CD94 antigen indicates their mature cell origin (99). Three types of leukemic cells were classified. Type I cells are LGLs, while type III cells are pleomorphic and atypical cells with prominent nucleoli. Type II cells show an intermediate morphology between types I and III (100). At presentation, this disease is sometimes indistinguishable from CLPD-NK, but from our experience, the presence of conspicuous nucleoli in NK cells strongly supports a diagnosis of ANKL. Furthermore, the diagnosis can be predicted by the following factors: <40 years of age, fever, lymph node swelling, and hepatosplenomegaly (15). Extranodal NK-cell lymphoma may also present as (or eventually develop) leukemic changes, and the distinction between ANKL and extranodal NK-cell lymphoma is sometimes difficult unless a nasal lesion is found. Few clinicopathological differences exist between younger NK-cell lymphoma and ANKL patients (101). The incidence of skin involvement is significantly higher among patients with extranodal NK-cell lymphoma (83). An array-based comparative genomic hybridization analysis demonstrated clear genetic differences between ANKL and extranodal NK-cell lymphoma, suggesting that these are two distinct diseases (102).

Oligo-array comparative genomic hybridization revealed that the most frequently deleted chromosomal regions in ANKL and extranodal NK-cell lymphoma were in 6q21, which includes tumor suppressor genes *FOXO3* and *PRDM1*, which may be involved in the pathogenesis of NK-cell neoplasms (103). In most patients, clonal EBV is found in tumor cells, and EBV is considered to be the etiological agent (89, 104, 105). Rare cases of EBV-negative ANKL have been described (106). It tends to occur in older patients and is clinically and pathologically indistinguishable from EBV-positive ANKL, with a similar fulminant clinical course. The high prevalence of Asian patients seen with EBV-positive disease seems less evident with EBV-negative cases.

The disease is refractory to chemotherapy and exhibits a

relentless progressive course with a poor outcome (107-109). The types of chemotherapy that should be used, and whether HSCT should be combined are matters of great concern (107-111). As in extranodal NK-cell lymphoma, this poor outcome may be partially explained by the presence of P-glycoprotein (P-gp), a multidrug resistance gene-encoded protein on the NK-cell membrane, which extrudes various cytotoxic agents, such as vinca alkaloids and anthracyclines (112-114), and combination chemotherapies using P-gp-unrelated drugs are expected. As an initial therapy, L-asparaginase chemotherapy resulted in a better response. A retrospective Japan-Korea multicenter study (100) was conducted to better elucidate the clinicopathological features and therapeutic modalities for ANKL. A total of 34 patients were analyzed. Six patients received allogeneic HSCT and two received autologous HSCT, all of whom were in non-complete remission. After HSCT, 4 patients who received allogeneic HSCT and one who received autologous HSCT reached a complete remission. The median survival of all patients was 51 days. The median survival of the patients who were treated with and without HSCT was 266 and 36 days, respectively. Two patients who received allogeneic HSCT were alive and in complete remission. All patients without HSCT died of ANKL. The use of L-asparaginase was indicated as a factor for longer survival. An early diagnosis, treatment with L-asparaginase-based chemotherapy, and allogeneic HSCT might lead to improved patient outcomes. A Korean study also supported the above findings (115). In one patient, when ANKL relapsed after allogeneic HSCT, cyclosporine was rapidly tapered and chemotherapy was initiated. During hematopoietic recovery, the number of atypical lymphocytes increased, and they were found to be donor-derived EBV-specific CTL. The patient achieved a partial response and the EBV viral load decreased to the normal range (116). EBV-reactive immunotherapy may be promising.

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## Future Prospects

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First, by increasing our understanding about the nature of T-LGL leukemia, we will recognize whether or not T-LGL leukemia is a true hematologic malignancy. Based on the findings, the name "T-LGL leukemia" may be changed to something more appropriate. Second, the diagnostic criteria of T-LGL leukemia should be more sophisticated. Third, further investigations are required to find the primary event of LGL proliferation, and the commitment of molecular changes to allow LGL expansions. Fourth, the treatment of T-LGL leukemia remains unsatisfactory, and molecularly targeted therapy should be extensively investigated in this era of precision cancer medicine. ANKL is very difficult to treat. This is mainly due to our limited knowledge on the molecular events leading to the development and progression of the disease. Extensive investigations are required.

**Author's disclosure of potential Conflicts of Interest (COI).**

Kazuo Oshimi: Advisory role, Eisai.

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